Blood sampling is essential for measurement of hormones in animal experiments. Sampling often gives pain to animals, and causes stress. Blood levels of some hormones are influenced by such stress. In order to avoid such possibility, blood sampling is very often carried out under anesthesia, however, anesthetics may also cause changes of blood levels of target substances. Blood sampling from conscious, free-moving atrial-cannulated animals is most suitable. But the atrial cannulation via jugular vein is not always practical.

The author surveyed some literatures reporting the effect of stress and/or anesthetics on blood levels of hormones, hoping that these data would be of some help to our ELISA kits users.

**Effects of stress**

**Cold exposure stress**

Cold exposure is often carried out by moving animals from room temperature or 30°C circumstances to 4°C cold room.

- After 5 minutes’ exposure (1)
  - Prolactin: increased to twice.
  - GH: significantly decreased from 295 to 128 ng/ml.
  - Corticosterone: significantly increased.

- Cold exposure (4°C) for 30, 60 minutes (2)
  - TSH: increased
  - This increase was fully suppressed by ether, urethane, and chloral hydrate anesthesia.
  - Thiopental partially suppressed this increase, but pentobarbital inhibited not significantly.
    - Halothane and methoxyflurane retarded the increase.

- Twenty five minutes after cold exposure (11)
  - TSH: Upraised from basal level of 0.1μg/ml to 0.49μg/ml.

**Immobilization (Restraint) stress**

Animals are fixed on plates or placed in small restraining cages.

- Immobilization stress for 5 minutes (1)
  - Prolactin: increased to 10 fold
  - GH: lowered significantly
  - Corticosterone: increased significantly

- Eight hours’ immobilization for 10 days (adult female rats) (7)
  - Plasma levels
    - GH, Prolactin, LH, TSH: lowered
    - FSH: lowered slightly but not significantly
Pituitary contents
- FSH, LH: increased
- GH, prolactin, TSH: no change
TRH, GnRH contents in the hypothalamus: no change
Intravenous injection of GnRH+TRH to such rats
- LH, TSH, FSH: secretory reactions were enhanced
- Prolactin: upraised
- GH: further decreased than before injection

Changes in pituitary hormone blood levels induced by in chronic stress are not due to the changes of pituitary reactivity to hypothalamic releasing hormones nor related to the changes in hypothalamic releasing hormones. The secretory responses of LH and FSH to releasing hormone may be secondary phenomenon due to the increases of their pituitary contents (7).

- Serum levels during immobilization (9)
  - Prolactin: increased at 10 minutes, and then fell down
  - TSH: slightly lowered at 10 minutes, and then decreased to a half, and further decreased to 1/2 at 300 minutes.
  - Repeated 10 minutes' immobilization
    - Prolactin increased when fixed and lowered when released. With repetition, the upraised levels went smaller.
  - Hypodermic injection of 0.2ml formalin after 300 minutes' immobilization caused an increase of prolactin after 10 minutes, and then fell down. Without immobilization, formalin injection failed to upraise prolactin level.
  - Regardless of 300 minutes' immobilization, an intravenous injection of 0.63mg/kg of Pimozide (a dopamine receptor blocker) was increased and reached a peak at 10 minutes, and kept the high level thereafter.
  - TRH injection after 300 minutes' immobilization
    - Prolactin: further increased after 10 minutes. Effect of TRH was not observed without immobilization.
    - TSH: regardless of immobilization, increased after 10 minutes, and then decreased to 1/2 after 30 minutes.
  - Pimozide pretreatment (20minutes) before immobilization
    - Prolactin: increased by pretreatment, and lowered at 10 minutes after immobilization was started, and the level increased again 60minutes' later and stayed high throughout immobilization.

- In conscious rats caged in restraint cages, secretory rate of testosterone was 4.2 ± 0.6 ng/g testis/min, while in halothane-anesthetized rats the rate was 6.7 ± 1.2 ng/g testis/min. The lower testosterone secretory rate in restrained conscious rat may be due to stress factor. (20)

Inescapable electric footshock (3)
- Prolactin: increased
- β-Endorphin: increased
- ACTH: increased
These increases were blocked by dexamethasone pretreatment.
Dexamethasone also decreased basal levels of prolactin, ACTH, and β-endorphin.

**Fasting (Starvation)**

By fasting, (10)
- LH: decreased significantly
- FSH: decreased significantly
- Testosterone: decreased significantly

Following the fall in blood glucose level, insulin was decreased and GH was increased. Refer to section of Ketamine (21).

**Effects of Ether**

- TSH: decreased in both light and deep anesthesia. At 30 minutes’ slight anesthesia: p<0.05, and deep anesthesia: p<0.01 (2). Ether anesthesia decreased the slope of dose-response curve of TRH-induced TSH release. Ether anesthesia during cold exposure completely blocked the increase of TSH caused by cold exposure. Ether anesthesia made at 3 minutes before cold exposure suppressed and retarded the TSH increase caused by cold exposure. (11)

- Prolactin: increased. Blocking of dopamine receptor by pimozide increased prolactin level. Ether anesthesia caused further increase (4).

Ether stress in male rats caused an increase of prolactin from 10ng/ml to 40ng/ml, and in male rats given estradiol from 100ng/ml to 400ng/ml (5)

Ether stress is considered to increase prolactin level via PRF (5).

Ether anesthesia increased serum prolactin level after 2.5 minutes, and 5 minutes later, this level became a half. In males, Prolactin basal level increased to twice in males and 4 times in females after 5 minutes of ether anesthesia (6).

- Ether inhalation in ovariectomized rats (8)
  - Prolactin, LH, FSH: increased within 2 minutes.
  - The order of the increase: prolactin>LH>FSH

Following the second treatment by ether,
  - Prolactin: further increased.
  - LH, FSH: showed no further increase.

These increased levels of prolactin. LH and FSH went down to less than those of controls (non-stress, decapitated) within 1 hour.

- One minute’s ether inhalation after 300 minutes’ immobilization increased serum prolactin level with a peak at 5 minutes, and thereafter the high level decreased. Without immobilization, prolactin did not change (9).

- Put rats in an ether container, and took them out as soon as they became unconscious, and decapitated. LH and FSH increased significantly, while testosterone did not change (11).

- Treatment female rats with ether 3 times at diestrus increased prolactin twice the level but LH did not change. The same ether treatment at proestrus decreased prolactin level by half and doubled LH level (16).

- In ovariectomized golden hamster (ovariectomized 3 weeks before experiment, and LH
level had reached 400–600 ng/ml), deep ether anesthesia increased LH level 30 to 60 minutes later (19).

- *In vitro* experiments: Ether inhibited glucose-dependent insulin release from the piece of pancreas in dose-dependent manner (29).

**Effects of various anesthetics**

**Pentobarbital sodium**

- Pentobarbital did not lower TSH level at room temperature, but inhibited upraise of TSH level by cold exposure by 90%.
  The slope of dose-response curve of TRH was enlarged by pentobarbital. In pentobarbital treated rats TSH secretory effect of TRH was larger than in controls (2, 11).
- Serum prolactin level was raised significantly at 30 minutes after intraperitoneal injection of pentobarbital. This increase was more evident in females (more than 10 times) than in males (3~7 times) (6).
- Administration of pentobarbital to female rats on the early afternoon of proestrous day caused an increase of prolactin level for 30 minutes, and completely blocked prolactin and LH surges in the late afternoon. Pentobarbital (3 μg) incubated with pituitary gland inhibited prolactin release (12).
- Intraperitoneal injection (40mg/kg) of pentobarbital to female rats in early afternoon (13:20, critical period) blocked preovulatory LH surge and also ovulation. After the injection, prolactin level was increased rapidly, but the spontaneous preovulatory prolactin surge was blocked (18).
- In golden hamsters, administration of pentobarbital in the early afternoon on proestrous day (so-called critical period) did not block ovulation. In hamster, ether anesthesia for 2-5 hours in the early afternoon on proestrous day did not block ovulation, however, it retarded nervous stimulation for ovulatory LH surge. In hamster, ovulatory LH surge occurs at 14:00~15:00, however, ether or pentobarbital anesthesia made at 13:00~15:00 retarded the first rise of LH by 1 hour. Ether anesthesia did not change the highest level of LH surge. On ther other hand pentobarbital anesthesia lowered the height of the peak (13).
- Administration of pentobarbital in the morning of estrous day followed by stimulation of cervix uteri caused blood LH and prolactin levels and inhibited induction of pseudo-pregnancy (14). LH and prolactin levels stayed low without the cervical stimulation. Without pentobarbital injection, cervical stimulation caused pseudo-pregnancy (15).
- Intra-arterial injection of L-DOPA (25mg/kg) to female rats at 13:00 of proestrous day caused a rapid increase of LH level. Pentobarbital anesthesia carried out at 12:30 blocked rapid LH increasing action of L-DOPA. This dose of L-DOPA did not increase LH level in diestrous rats (16).
- Pentobarbital inhibited the increase of prolactin level caused by ether anesthesia. Pentobarbital completely inhibited the increase of prolactin level caused by a'-methyl-m-tyrosine (an inhibitor of catecholamine synthesis). Pentobarbital inhibited the increase of prolactin level caused by estrogen treatment.
Pentobarbital, within 10 minutes, lowered the upraised prolactin level during ether anesthesia in ovariectomized rats. This lowered prolactin level continued for at shortest 120 minutes (17).

- Deep anesthesia by pentobarbital sodium (8mg/100g, intra-peritoneal injection) lowered LH level tentatively in ovariectomized hamsters which had been operated 3 weeks before anesthesia showing high LH levels (19).
- Pentobarbital sodium did not cause hyperglycemia in both fasting and fed rats (21).
- Pentobarbital did not change plasma glucose level, however, increased insulin level (from 0.59ng/ml to 2.13ng/ml) (38).

**Thiopental sodium (Sodium pentothal)**
- Effect of TRH on TSH secretion was larger in thiopental anesthetized rats than in controls (2).
- Intra-arterial injections of sodium pentothal (20mg/kg, 3 times) to female rats at critical period on proestrous day blocked preovulatory surges of LH and prolactin and ovulation, however did not increase prolactin level immediately after administration different from pentobarbital (18).
- In dogs, intravenous glucose tolerance test during infusion of thiopentone indicated increased insulinogenic index. Hyperglycemia seemed to promote insulin secretion (36).

**Phenobarbital**
- Phenobarbital did not induce anesthesia in ovariectomized hamster (operated 3 week before the experiment and blood LH levels reached 400~600ng/ml), however it decreased LH levels far more than pentobarbital, indicating that LH decreasing action has nothing to do with anesthetic activity (19).

**Ketamine**
- Ketamine (100mg/kg) was administered to fed and fasting SD rats together with xylazine (muscle relaxant, analgesic, and sedative agent, 10mg/kg).
  In rats fed ad libitum administration of drugs induced acute hyperglycemia (178.4 ± 8.0mg/dl) within 20 minutes, and at the same time, it caused a decrease in insulin, ACTH and corticosterone levels. Glucagon and GH levels were increased. This hyperglycemia was blocked by yohimbine (1~4mg/kg) in dose-dependent manner.
  K+X might stimulate α2-adrenergic receptor and changed glucose metabolism hormones
  Ketamine alone failed to cause hyperglycemia.
  In fasting rats, K+X administration did not cause acute hyperglycemia.
  These facts indicated that we have to select anesthetics, and take the feeding states of animals into consideration in experimental models where diabetes, glucose and levels of glucose metabolism-related hormones influence the experimental results (21).

**Chloral hydrate**
- Chloral hydrate enhanced the TSH-secretory effect of TRH in rats (2).

**Isoflurane**
- Isoflurane anesthesia caused acute hyper glycemia within 20 minutes in rats fed ad
In IVGTT (intravenous glucose tolerance test) in Japanese white rabbits, isoflurane anesthesia (1.0 minimum alveolar concentration) made 30 minutes before glucose injection (0.6mg/kg) lowered insulinogenic index \( \frac{\text{increase in insulin (μU/ml)}}{\text{divided by increase in glucose level(mg/dl)}} \). Isoflurane may inhibit insulin secretion and glucose utilization (22).

I Isoflurane (2%) lowered insulin secretion from isolated Langerhans islets caused by glucose (20mmol/l) to basal non-stimulated levels. It also suppressed insulin release induced by glyceraldehydes and phorbol ester. It was suggested that this inhibitory effect may act beyond glyceraldehydes and phorbol ester (23).

In blood sampling from male dwarf hamster (Phodopus spp) by holding with hand without anesthesia, upraise of cortisol and decrease of prolactin levels at 1 minute later. Such changes were not observed when caged animals were treated with isoflurane (24).

In human appendix operations, the marked increases of cortisol and T4 were also observed under isoflurane anesthesia. Isoflurane failed to block the increase of these hormones which occurs with surgical operation (25).

Hematology and blood chemistry data of rats anesthetized with isoflurane were nearly the same to those of ether anesthesia except slight change in Ca ion concentration. Blood prolactin and cortisosterone levels indicated that isoflurane anesthesia gave smaller stress than ether (26).

Isoflurane decreased RBC, hemoglobin, and hematoclit in female rats, and increase glucose level in males. It decreased chloride, serum protein, sodium, inorganic phosphate, calcium, magnesium, and potassium, T3 and T4, and increased prolactin. It did not affect erythrocyte cholinesterase activity, and serum and brain cholinesterase activity. Isoflurane is an anesthetics of choice (27).

Isoflurane rapidly and widely influenced on metabolism. Ten minutes’ anesthesia by isoflurane decreased the metabolism of free fatty acid and palmitate, increased gluconeogenesis, and anesthesia for 3.5 hours lowered peripheral glucose utilization. It increased periphery insulin resistance and lowered fat degradation, and in parallel with the term of anesthesia, it decreased protein synthesis and increased the rate of amino acid oxidation (28).

IVGTT carried out under isoflurane anesthesia in human subjects showed that insulinogenic index, acute insulin response and glucose disappearance rat were lowered than in non-anesthetized controls (37).

Isoflurane casuse hyperglycemia in rats fed *ad libitum*, but it did not affect insulin level. The hyperglycemia caused by isoflurane was blocked by glibenclamide (non-specific K(ATP) channel inhibitor). Glucose-dependent insulin secretion might be possible interfered by isoflurane (38).

**Halothane**

- Four hours’ general anesthesia by halothane, in dogs, caused a decrease in protein biosynthesis and enhancement of leucine oxidation rate (28).
- Halothane interfered with *in vitro* glucose-dependent insulin release from pancreatic fragment in dose-dependent manner (30).
- Halothane interfered in dose dependent manner with glucose-dependent release of
insulin from isolated islets of Langerhans. This action was not caused by glucose oxidation inhibition (31).

- When compared in the same pony with and without 2 hours' halothane anesthesia, halothane anesthesia (without surgical invasion) increased plasma glucose, lactate, cortisol and ACTH levels, and lowered insulin level. These results were confirmed in a similar experiment carried out 18 months later. It was shown that halothane caused practically stress reaction in horse (32).

- Insulin injection to starved lactating rats increased pyruvate dehydrogenase activity in mammary gland, but not pyruvate dehydrogenase activity in the liver. This action of insulin was markedly inhibited by halothane anesthesia (33).

- Halothane anesthesia for 24 hours decreased glucose utilization rate by 20% in rats. This was a similar to natural sleep, not specific to halothane. Among rats under halothane anesthesia, blood insulin concentration was negatively correlated to rate coefficient of glucose utilization (34).

- Halothane anesthesia was induced to rats about 70 minutes after non-lethal scald injury rapidly raised plasma glucose concentration during and shortly after induction, which by 30 minutes began to return to the values in injured controls. Insulin concentration was also increased. Anesthesia for 2 hours lowered glucose production and utilization, and increased plasma insulin level, and decreased liver glycogen content, i.e. it had exacerbated well-known effects of injury in rat, including insulin resistance (35).

- IVGTT in dogs under halothane anesthesia showed that halothane lowered insulinogenic index significantly (36).

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